ONTARIO PORK

Ontario Pork Research Final Report (#055157) Executive Summary

Reporting Date: 31/08/2022

Introduction: There is currently no reliable, non-invasive technique to predict the chemical composition of skeletal muscles in live animals. We hypothesized that ultrasound imaging combined with computer-assisted analysis would be a suitable method to assess the chemical composition of skeletal muscles in fatteners.

Objectives: To employ commercially available image analysis software (e.g., ImageProPlus® or ImageJ) for a rapid determination and/or prediction of chemical composition and physical properties of skeletal muscles in pigs.

Materials and Methods: Ultrasonograms of the pigs' longissimus dorsi muscles were obtained just before slaughter, using the Aloka PS2 ultrasound scanner (Aloka Ltd., Tokyo, Japan) connected to a hand-held 5.0-MHz of 7.5-MHz linear-array transducer. All echotextural analyses were subsequently conducted at the University of Guelph, ON, Canada, and utilized the ImageProPlus®7.0 analytical software. Chemical composition and physical properties of muscle samples were determined by the diagnostic laboratory in the OAC. All of the methods used have been tested and validated in our previous studies (Schwarz et al., *Animals* 2019;9:306).

Results and Discussion: Significant correlations with echotextural attributes were recorded for different chemical constituents and physical properties of the longissimus dorsi muscles from fatteners receiving different amounts of corn in their diets, but none of the correlation was observed consistently in all subsets of experimental animals (nutrition groups).

Conclusions: There exists a potential application for ultrasonographic imaging in situ combined with computerized image analysis to estimate/predict certain chemical constituents, physical characteristics of muscles and sensory meat attributes in live pigs. However, the existence and strength of correlations among ultrasonographic image attributes and muscle composition must be determined using analyses of the processed images to maintain the consistency of outcomes.

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Introduction: There is currently no reliable, non-invasive and inexpensive technique to evaluate and predict the chemical composition of skeletal muscles in live animals. Chemical composition of meat can only be accurately measured post-mortem, which hampers genetic selection of meat-producing animals. Biochemical content of skeletal muscle can be evaluated in live animals only through biopsies that cannot be performed frequently. The main goal of the proposed study was to employ grey-scale ultrasonography and image analysis to determine if quantitative echotextural attributes of skeletal muscle echotexture are associated with changes in their chemical constituents in fatteners. This approach could then be used for studying various aspects of animal nutrition, genetic selection and an array of other production strategies aimed to improve meat quality.

Objectives: 1. To develop and optimize the technique for acquisition of high quality ultrasonographic images of skeletal muscles (e.g., longissimus dorsi or semimembranosus) in live pigs using portable ultrasonographic equipment with a linear array probe and sufficient recording ability or connected to light-weight computers; 2. To employ commercially available image analysis software (e.g., ImageProPlus® or ImageJ) for a rapid determination and/or prediction of chemical composition and physical properties of skeletal muscles in pigs; 3. In a preliminary assessment, evaluate the usefulness of ultrasonography for predicting the final meat quality traits (physical and chemical attributes).

Materials and Methods: The University of Guelph Animal Care Committee reviewed all of the animal handling and experimental procedures as well as animal slaughter protocols. The entire fattening period (approx. 110 days) of 30 cross-bred fattener pigs consisted of the grower and finisher phases (approx. 65 and 45 days for the grower and finisher phase, respectively). Ultrasonograms of the pigs' left longissimus dorsi and semimembranosus muscles were obtained just before slaughter, using the Aloka PS2 ultrasound scanner (Aloka Ltd., Tokyo, Japan) connected to a hand-held 5.0-MHz of 7.5-MHz linear-array transducer. Each muscle was scanned in a transverse and longitudinal plane, and still images containing the largest cross-sectional areas of the muscle were saved as digital (DICOM) images with the resolution of 640 x 480 pixels. All echotextural analyses were subsequently conducted at the University of Guelph, ON, Canada, and utilized the ImageProPlus®7.0 analytical software (Media Cybernetics Inc., Rockville, MD, USA). Chemical composition and physical properties of muscle samples were determined by the diagnostic laboratory in the OAC. Fatty acid composition of longissimus dorsi (gas chromatography) was analyzed in licensed analytical laboratories. All of the methods used have been previously tested and validated in our previous studies (e.g., "Quantitative echotextural attributes of pectoralis major muscles in broiler chickens: Physicochemical correlates and effects of dietary fat source" by Schwarz et al., *Animals* 2019;9:306).

Results and Discussion: Significant correlations with echotextural attributes were recorded for different chemical constituents and physical/sensory properties of the longissimus dorsi muscles from fatteners receiving different amounts of corn in their diets (0-60%), but none of the correlation was observed consistently in all subsets of experimental animals (nutrition groups). See tables below for the details of correlations among ultrasonographic and laboratory results, with all the data presented for separate groups as well as all fatteners studied (pooled results).

Table 1. Summary of significant correlations between echotextural characteristics of longissimus dorsi and its chemical constituents/physicochemical characteristics in fatteners receiving different proportions of corn added to the compound diet and examined before slaughter with the 7.5-MHz linear-array transducer (n=96). PI-pixel intensity or brightness; SD-pixel standard deviation or heterogeneity; Longitudinal, Transverse-scanning planes.

Input variable (x)	Output variable (y)	r	P value	Regression equation
Longitudinal PI	**Group 0% (corn content in the compound diet)**			
	pH 45min	0.65	0.02	y=5.83+0.02x
	pH 24hr	0.85	0.0005	y=9.18-0.20
	Group 20%			
	16:0	0.69	0.03	y=27.61-0.18x
	SFA	0.70	0.02	y=47.63-0.34x
	Group 60%			
	L*	0.79	0.004	y=52.96-0.10x
Transverse PI	**Group 0%**			
	pH 45min	0.69	0.01	y=5.77+0.03x
	Group 20%			
	pH 45min	0.67	0.03	y=5.82+0.02x
	Group 40%			
	Crude fat	0.72	0.01	y=2.97-0.06x
	20:3 n3	0.63	0.04	y=0.16-0.001x
	Group 60%			
	Color	0.70	0.02	y=2.53-0.02x
	Texture	0.65	0.03	y=2.88-0.04x
	Water absorbance	0.68	0.05	y=37.65-0.18x
Longitudinal SD	**Group 0%**			
	Moisture	0.61	0.04	y=74.74-0.09x
	pH 24hr	0.89	0.0001	y=11.44-0.37x

	20:1	0.58	0.05	y=0.70+0.01x
	Group 20%			
	14:0	0.66	0.04	y=1.39-0.01x
	16:0	0.77	0.009	y=30.08-0.33x
	18:2	0.65	0.04	y=3.51+0.47x
	20:2	0.68	0.03	y=0.23+0.02x
	20:3 n3	0.71	0.02	y=0.08+0.003x
	SFA	0.75	0.01	y=51.79-0.58x
	PUFA	0.65	0.04	y=4.39+0.53x
	n6	0.65	0.04	y=3.85+0.51x
	Group 40%			
	18:0	0.65	0.03	y=9.75+0.20x
	Group 60%			
	L*	0.66	0.03	y=54.36-0.18x
Transverse SD	**Group 0%**			
	pH 45min	0.58	0.05	y=5.58+0.04x
	20:3 n3	0.70	0.01	y=0.07+0.002x
	Group 20%			
	pH 45min	0.67	0.03	y=5.51+0.04x
	14:0	0.65	0.04	y=1.39+0.01x
	Group 40%			
	Texture	0.70	0.02	y=0.68+0.06x
	20:3 n3	0.65	0.03	y=0.17-0.002x
	Cholesterol	0.62	0.04	y=110.64-1.51x
	Group 60%			
	Texture	0.61	0.05	y=3.31-0.07x
	Color	0.63	0.04	y=2.71-0.03x

Table 2. Summary of significant correlations between echotextural characteristics of longissimus dorsi and its chemical constituents/physicochemical characteristics in fatteners receiving different proportions of corn added to the compound diet and examined before slaughter with the 7.5-MHz linear-array transducer. Data were pooled for all animals studied.

Input variable (x)	Output variable (y)	r	P-value	Regression equation
Longitudinal PI	Color	0.31	0.04	y=1.94+0.009x
	20:2	0.35	0.02	y=0.60+0.004x
Transverse PI	pH 45min	0.34	0.02	y=6.11+0.01x
	10:0	0.36	0.02	y=0.06-0.0003x
Longitudinal SD	L*	0.32	0.03	y=54.95-0.22x
	Exudate	0.44	0.04	y=10.15-0.24x
	10:0	0.33	0.03	y=0.06-0.0005x
	20:2	0.30	0.05	y=0.55+0.008x
Transverse SD	pH 45min	0.30	0.04	y=6.00+0.02x
	10:0	0.32	0.03	y=0.06-0.0005x

Table 3. Summary of significant correlations between echotextural characteristics of longissimus dorsi and its chemical constituents/physicochemical characteristics in fatteners receiving different proportions of corn added to the compound diet and examined before slaughter with the 7.5-MHz convex-array transducer (n=96).

Input variable (x)	Output variable (y)	r	P value	Regression equation
Longitudinal PI	**Group 0%**			
	pH 45min	0.61	0.04	y=5.83+0.02x
	Group 20%			
	Aroma	0.67	0.04	y=3.76+0.005x
	pH 48hr	0.78	0.01	y=5.84+0.005x

	b*	0.75	0.01	y=-3.55+0.08x
	Group 40%			
	pH 48hr	0.76	0.01	y=5.42+0.003x
Transverse PI	Group 0%			
	18:2 c9 t11	0.66	0.021	y=0.09-0.002x
	20:1	0.75	0.005	y=0.61+0.01x
	Group 20%			
	pH 24hr	0.72	0.020	y=-1.47+0.21x
	b*	0.66	0.036	y=-5.66+0.16x
	Group 40%			
	Texture	0.68	0.021	Y=1.37+0.01x
Longitudinal SD	Group 0%			
	pH 24hr	0.63	0.03	y=13.08-0.43x
	Cholesterol	0.60	0.04	y=48.81+0.94x
	MUFA	0.64	0.03	y=38.74+0.26
	Group 20%			
	Texture	0.76	0.02	Y=2.81-0.05x
	Aroma	0.89	0.0009	Y=3.32+0.03x
	pH 45min	0.75	0.01	Y=5.33+0.05x
	L*	0.66	0.04	Y=33.94+0.80x
	b*	0.70	0.03	Y=-7.70+0.37x
	20:1	0.64	0.048	Y=0.67+0.02x
	Group 40%			
	20:3 n3	0.71	0.01	Y=0.18-0.002x
	Group 60%			
	Texture	0.76	0.0006	Y=4.37-0.10x
Transverse SD	Group 0%			
	Moisture	0.70	0.01	y=77.18-0.21x
	Protein	0.75	0.01	y=20.25+0.19x
	Group 20%			
	Taste	0.69	0.03	y=2.54+0.06x
	pH 45 min	0.66	0.04	y=5.06+0.06x
	18:3	0.71	0.02	y=1.30-0.03x
	20:1	0.67	0.03	y=0.47+0.03x
	22:5	0.64	0.049	y=0.16-0.004x
	n3	0.70	0.02	y=1.46-0.03x
	Group 40%			
	pH 24hr	0.65	0.03	Y=13.75-0.48x

Table 4. Summary of significant correlations between echotextural characteristics of longissimus dorsi and its chemical constituents/physicochemical characteristics in fatteners receiving different proportions of corn added to the compound diet and examined before slaughter with the 7.5-MHz convex-array transducer. Data were pooled for all animals studied.

Input variable (x)	Output variable (y)	r	P-value	Regression equation
Longitudinal PI	20:1	0.41	0.01	y=0.866+0.004x
	n3	0.35	0.02	y=0.936-0.003x
Transverse PI	Texture	0.32	0.03	y=1.52+0.011x
Longitudinal SD	pH 45min	0.36	0.02	y=5.83+0.027x
	15:0	0.35	0.02	y=-0.006+0.002x
	17:1	0.32	0.03	y=0.138+0.007x
Transverse SD	Texture	0.32	0.04	y=0.850+0.052x
	Protein	0.32	0.04	y=20.79+0.136x
	18:2	0.37	0.01	y=20.663-0.333x
	20:1	0.30	0.050	y=0.654+0.018x
	20:3 n6	0.40	0.01	y=0.160+0.003x

	20:4	0.38	0.01	y=0.445-0.009x
	PUFA	0.37	0.01	y=23.77-0.376x
	n6	0.37	0.01	y=22.31-0.356

Table 5. Summary of significant correlations between echotextural characteristics of longissimus dorsi and its chemical constituents/physicochemical characteristics in fatteners receiving different proportions of corn added to the compound diet and examined after slaughter with the 7.5-MHz convex-array transducer (n=96).

Input variable (x)	**Output variable (y)**	**r**	**P value**	**Regression equation**
Longitudinal PI	**Group 0%**			
	18:2	0.41	0.045	y=11.03+0.07x
	Group 40%			
	Cholesterol	0.44	0.03	y=65.22+0.33x
	Group 60%			
	Texture	0.43	0.04	y=1.20+0.02x
Transverse PI	**Group 0%**			
	Juiciness	0.41	0.04	y=4.37-0.01x
	Thermal Loss	0.45	0.02	y=39.06-0.15x
	20:3 n3	0.57	0.003	y=0.20-0.001x
	Group 20%			
	14:0	0.45	0.04	y=0.10+0.003x
	16:0	0.51	0.02	y=21.13+0.05x
	20:3 n3	0.45	0.04	y=0.16-0.0005x
	Cholesterol	0.42	0.050	y=88.73-0.24x
	Group 40%			
	18:3	0.51	0.01	y=1.00-0.006x
	20:3 n3	0.46	0.03	y=0.19-0.001x
	n3	0.50	0.01	y=1.09-0.006x
	n3/n6	0.41	0.046	y=0.08-0.0005x
	n6/n3	0.43	0.04	y=11.88+0.15x
	Group 60%			
	Crude Fat	0.47	0.02	y=0.81+0.02x
Longitudinal SD	**Group 0%**			
	18:2 c9 t11	0.51	0.009	y=0.10-0.005x
	20:2	0.42	0.04	y=1.03-0.02x
	n3/n6	0.44	0.03	y=0.01+0.002x
	n6/n3	0.46	0.02	y=30.42-0.68x
	Group 20%			
	b*	0.44	0.04	y=5.40-0.26x
	20:1	0.46	0.03	y=1.56-0.02x
	Group 40%			
	a*	0.44	0.03	y=8.87-0.16x
	17:0	0.41	0.046	y=0.62-0.01x
	18:1 Trans	0.46	0.02	y=0.34-0.008x
Transverse SD	**Group 0%**			
	Water absorbance	0.45	0.02	y=39.90-0.40x
	Cholesterol	0.51	0.009	y=91.72-1.14x
	Group 60%			
	pH 45min	0.46	0.02	y=7.17-0.03x
	pH 24hr	0.50	0.01	y=-0.003+0.23x
	Exudate	1.0	0.01	y=19.84-0.81x

Table 6. Summary of significant correlations between echotextural characteristics of longissimus dorsi and its chemical constituents/physicochemical characteristics in fatteners receiving different proportions of corn added to the compound diet and examined after slaughter with the 7.5-MHz convex-array transducer. Data were pooled for all animals studied.

Input variable (x)	Output variable (y)	r	P-value	Regression equation
Longitudinal PI	b*	0.22	0.01	y=0.54-0.03x
Transverse PI	20:3n3	0.28	0.007	y=0.16-0.0006x
	Cholesterol	0.20	0.049	y=80.04-0.14x
Longitudinal SD	pH 48hr	0.23	0.02	y=5.44+0.007x
	Exudate	0.31	0.03	y=9.40-0.19x
	L*	0.28	0.005	y=56.25-0.26x
	b*	0.21	0.046	y=1.70-0.11x

Conclusions: The present study demonstrates the potential applicability of computerized analysis of ultrasonograms for determining chemical composition, physical properties and sensory attributes of the longissimus dorsi muscle in fatteners, including crude fat and protein content as well as select fatty acids. A lack of consistent correlations across the four nutrition groups appears to be due mainly to the relative abundance of individual chemical constituents ("threshold levels or concentrations") and warrants further studies. There exists a potential application for ultrasonographic imaging in situ combined with computerized image analysis to estimate certain chemical constituents of muscles in live pigs. However, the existence and strength of correlations among ultrasonographic image attributes and muscle composition must be determined using echotextural analyses of the processed images to maintain the consistency of outcomes.

Knowledge Transfer: All raw data are available upon reasonable requests to the public, and especially all the members and affiliates of Ontario Pork. Due to the COVID-19 pandemic, our plans to present our findings at the University of Guelph Swine Research Day and/or London Swine Conference have not been successful. We are planning to do so during the upcoming meetings in 2023 as well as the International Forum on Animal Science and Veterinary Medicine (ASVMFORUM2023), which will be held in Vancouver, Canada during the period of August 24-26, 2023. Even tough all the graduate and undergraduate students will no longer be able to partake, it is important to emphasize that this project involved the extensive training of HQP (6 undergraduate research project students, 1 PhD and 1 MSc student, and 2 summer students). The present results paved the way to outlining the conceptual framework to graduate thesis and the next Ontario Pork grant application to be submitted this fall (LOI). Two research articles are currently being prepared for submission and two more will likely use the concepts/data generated during the present Ontario Pork-funded research projects.